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SIMULTANEOUS PRODUCTION OF TWO CAPSULAR POLYSACCHARIDES BY PNEUMOCOCCUS

I. PROPERTIES OF A PNEUMOCOCCUS MANIFESTING BINARY CAPSULATION*

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PLATES 32 TO 35

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The concept that a pneumococcal cell may produce more than one capsular antigen is not new. The possible occurrence of the phenomenon was considered as early as 1928 by Griffith (1), who described, in his original report of the transformation reaction, a strain of pneumococcus agglutinated specifically by antisera to two different pneumococcal types. In the same year, the serologic cross-reactivity between pneumococcus Type III and a strain classified later as pneumococcus Type VIII was recognized (2, 3). It was shown subsequently by Goebel (4), however, that the immunologic relationship between these two capsular types resulted from the presence in their respective capsular polysaccharides of a common chemical subgroup rather than from the production by each type of several discrete capsular antigens, one of which was common to both. Similar immunologic cross-reactivity has been demonstrated among a number of other pneumococcal types (5), but in no instance has it been shown conclusively that an organism of this species produces more than one molecular type of capsular polysaccharide.

In 1953, Leidy, Hahn, and Alexander (6) reported on the production *in vitro* of new types of *Hemophilus influenzae* obtained by transforming capsulated or non-capsulated organisms of this species with deoxyribonucleates (DNA) derived from bacterial cells of heterologous capsular type. Organisms reacting with each of two unitypic anticapsular sera were isolated and, although definitive experiments are not described, it is possible that these cells were producing capsular polysaccharides of two molecular species. Analogous experiments performed with pneumococcus have led to the isolation of a variety of phenotypes manifesting binary capsulation. In this and in subsequent reports, some of their biological, biochemical, and genetic properties will be set forth.

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Materials and Methods

Nomenclature of Pneumococcal Variants.—In certain of the pneumococcal strains to be described, there is lack of complete correspondence between phenotype and genotype. For this reason, complexities arise in the description of these strains. For the present, only phenotypic designations will be employed and terms descriptive of genotype will be introduced later.

Fully capsulated phenotypes are indicated by the letter S followed by a Roman numeral designating capsular type; *e.g.*, SIII is a capsulated strain of pneumococcus Type III. Because of certain biochemical relationships to be described subsequently and for convenience, non-capsulated variants and intermediate capsular variants agglutinable by antibody to somatic antigens, including those described by Taylor (7) under the term SIII-1, will be designated by the expression $S-X_1$, the subscript designating the capsular type from which the mutant strain was derived and the strain's designation; *e.g.*, $S-III_4$ is Strain 4 of a non-capsulated phenotype derived from pneumococcus Type III. Phentotypes manifesting binary capsulation will be indicated by the letter S followed by the two Roman numerals descriptive of the strain's capsular components separated by a hyphen; *e.g.*, SI-III is a pneumococcus characterized by the production of both Type I and Type III capsular polysaccharides.

Strains of Pneumococcus.—SI phenotypes—SVI, ID, I41S, IP: strains of the SI genotype isolated originally from human sources and carried in the laboratory for several years.

SIII phenotype—IIIA66: a strain of the SIII genotype carried in the laboratory for many years.

$S-III$ phenotypes— $S-III_1$, $S-III_2$: strains of a non-capsulated variant of pneumococcus Type II (R36A) which carry different mutated Type III genomes acquired by transformation with DNA from variants of Strain IIIA66.

$S-III_3$: the same non-capsulated mutant of pneumococcus Type II transformed with DNA of a variant of the Type III Strain SV3. (The three preceding strains are Ephrussi-Taylor's SIII-1, SIII-1b, and SIII-1c (8) and were made available to us by her and by Dr. Arnold Ravin.)

$S-III_4$: a non-capsulated variant of strain IIIA66.

Preparation of DNA (Transforming Principles) and Technique of Transformation Reactions.—The methods used were those described by MacLeod and Krauss (9).

Preparation of Anticapsular Sera.—Vaccines of unitypic pneumococci and of pneumococci producing two capsular polysaccharides were prepared from cultures grown at 37°C. for 12 to 16 hours. After standing for 1 hour at room temperature following the addition of neutralized formalin to a final concentration of 1 per cent, the organisms were collected by centrifugation and resuspended in a volume of normal saline solution one-tenth that of the original culture. The suspension was heated at 65°C. for 30 minutes and stored at 4°C.

Rabbits were immunized by the injection intravenously of 1 cc. of vaccine daily for 5 days and were bled on the 10th day following each course of vaccine.

Precipitin Tests.—Precipitin tests were carried out in small test tubes. 0.2 cc. of each of several serial dilutions of antigen was layered over 0.2 cc. of serum. The tubes were incubated for 1 hour at 37°C. and the reactions read after the tubes had stood overnight at 4°C.

Precipitin reactions in agar gels were carried out according to the technique of Ouchterlony as modified by Halbert (10).

Virulence and Protection Tests.—Tests of virulence were performed by the injection intraperitoneally into mice of the CFW strain of 1 cc. of an appropriate dilution of a blood broth culture of the strain to be tested which had been incubated for 16 hours. Counts of viable bacterial units were obtained by dilution and plating. In protection tests, mice were injected intraperitoneally with 1 cc. of the serum to be tested 24 hours before being infected with pneumococci as in tests of virulence. Control animals received normal rabbit serum. All tests

were continued for 10 days and results were scored in terms of survival or death. The cause of death was determined by culture of the animal's blood at autopsy. In experiments in which the enzyme which hydrolyzes specifically Type III capsular polysaccharide[1] was employed, 2 mg. of enzyme were injected intraperitoneally.

Tests of Phagocytosis by Human Polymorphonuclear Leukocytes.—The technique employed was one described previously (11). When the enzyme hydrolyzing Type III polysaccharide was used, it was present in a final concentration of 2 mg./cc.

EXPERIMENTAL

Phenotypes Manifesting Binary Capsulation.—When cells of pneumococcal Strain S—III_4 are transformed in the presence of DNA from any of the Type I pneumococcal strains listed above, organisms of two different phenotypes can be recovered: cells of the SI phenotype and cells of the SI-III phenotype.

TABLE I

Relationships of Pneumococcal Strains Participating in Transformation Reactions Giving Rise to Variants Producing Two Capsular Polysaccharides

Original capsular type of strain manifesting the SI-III phenotype following transformation	Parent Type III strain of variant with S-III genome	Source of SI genome
SI	IIIA66	SVI
SII	IIIA66	SVI, ID, I41S, IP
	IIISV3	SVI, ID
SIII	IIIA66	SVI, ID, I41S, IP
SV	IIIA66	ID

In similar fashion, SI-III strains may be obtained by transforming cells of lines S—III_1, S—III_2 and S—III_3. From these experiments, it can be seen that mutant Type III cells, the capsular genomes of which were derived originally from two different Type III strains, IIIA66 and IIISV3, can be transformed to the SI-III phenotype with DNA from several SI strains. In addition, it is evident that non-capsulated cells derived originally from capsulated strains of different capsular types, *i.e.* Type II and Type III, may manifest binary capsulation when endowed with the proper genetic factors. It has been possible also to transform non-capsulated cells derived from Type I and from Type V pneumococci to the SI-III phenotype. The phenomenon of binary capsulation, therefore, may be expressed by cells derived from a variety of capsular types when they are endowed with the appropriate genetic factors derived from any of several Type I and mutant Type III strains (Table I).

[1] The enzyme preparation was made available through the generosity of Dr. Alan W. Bernheimer, Department of Microbiology, New York University College of Medicine.

SI-III cells were observed first in experiments in which strain $S-_{III_4}$ had been exposed to DNA from a capsulated Type I strain. In quellung preparations,[2] it was noted that the transformed cells showed capsular swelling when exposed either to Type I or to Type III antiserum. The size of the refractile capsular halo in Type III antiserum was approximately that seen about cells of the normally capsulated Type III phenotype but the refractile zone about cells in Type I antiserum was distinctly smaller than that about capsulated Type I cells and at times could be seen only with difficulty. Variation in the size of the two capsular components in relation to the phase of bacterial growth was not observed. When the cells manifesting binary capsulation were exposed to a mixture of Type I and Type III antisera, the capsular swelling was not recognizably greater than that which followed contact with Type III antiserum alone. In smears stained by the Gram technique, such cells showed no consistent variation in size or in shape which permitted their distinction from singly capsulated variants. SI-III cells isolated from the peritoneal cavity of the white mouse did show elongated forms often in short chains, morphologic characteristics not manifested by the organism when grown *in vitro*.

On solid medium, SI-III cells give rise to clones morphologically indistinguishable from those formed by pneumococcus Type III, a not surprising finding in view of the significant amount of Type III polysaccharide formed by the SI-III cell.

Evidence Establishing the Existence of the SI-III Cell.—The observation that more than 90 per cent of cells from clones of the SI-III phenotype gave a positive quellung reaction in both Type I and Type III anticapsular serum provided strong presumptive evidence for the existence of a cell producing two capsular polysaccharides or one so constituted chemically as to possess the serologically reactive groups of Type I and of Type III polysaccharide.

To distinguish between cells manifesting binary capsulation and mixtures of SI and SIII cells, several types of experiment were performed. Table II shows the results of growing SI, SIII, mixtures of SI and SIII cells and SI-III cells in broth containing either Type I or Type III anticapsular serum. Only those cells producing simultaneously two capsular polysaccharides are agglutinated by both unitypic antisera and yield cultures with limpid supernatant fluids in the presence of each antiserum in so called "thread tests."

Similar evidence distinguishing cells with binary capsules from unitypic cells has been obtained from mouse protection tests. The evidence in Table III indicates that all animals infected with cells with binary capsules were protected by either unitypic capsular antiserum whereas none was protected by either

[2] Capsular typing sera were made available through the courtesy of Dr. H. D. Piersma, Lederle Laboratories Division, American Cyanamid Company, and Dr. E. F. Roberts, Wyeth Laboratories, American Home Products Corporation.

antiserum when infection was carried out with mixtures of Type I and Type III cells.

Evidence for the Production of Two Capsular Polysaccharides by SI-III Cells.— SI-III cells grown by continuous passage in broth containing either Type I or Type III anticapsular serum give rise to mutant cells producing in amounts detectable by the quellung reaction only Type III or Type I capsular polysaccharide. These mutants derived from SI-III cells suggest that separately variable factors controlling two capsular components are present in the SI-III cell but do not exclude the possibility that each mutant is associated with a qualitative variation in the composition of one of two polysaccharides.

A second type of evidence is derived from the exposure of SI-III cells to the enzyme which hydrolyzes Type III capsular polysaccharide. After such treatment, SI-III cells are no longer agglutinable by Type III anticapsular serum and they fail to give a quellung reaction with Type III antibody. Their agglutinability by Type I anticapsular serum remains unimpaired, however, and the treated cells give still a positive quellung reaction with Type I antibody. It has been shown that the action of the enzyme hydrolyzing Type III pneumococcal polysaccharide is more highly specific than antibody to Type III polysaccharide. In addition, the action of this enzyme has been demonstrated recently to be that of an endoenzyme rather than that of an exoenzyme attacking the end-groups of a macromolecule (12). The experiments suggest strongly, therefore, that the SI-III cell produces normal Type III polysaccharide or a substance resembling it very closely and one that is probably distinct from the Type I component of the capsule.

TABLE II

Appearance of Supernatant Fluid of Pneumococcal Cultures Containing Unitypic Anticapsular Serum

Pneumococcal type or types in culture	Serum	
	Anti-SI	Anti-SIII
SI	L	T
SIII	T	L
SI + SIII	T	T
SI-III	L	L
SI-III + SI	L	T
SI-III + SIII	T	L

Medium: neopeptone broth plus 10 per cent anticapsular rabbit serum; L = limpid supernatant fluid. T = turbid supernatant fluid.

In precipitin reactions carried out in an agar gel by the modified technique of Ouchterlony (10), the precipitates formed when Type III anticapsular

antibody is allowed to react in the same plate with SI-III cells, SIII cells and Type III polysaccharide give rise to a line of continuity (Fig. 1); and, in like manner, a line of continuity is produced when the reactants are Type I anticapsular antibody, SI-III cells and Type I polysaccharide (Fig. 2).

Precipitin tests performed with partially purified polysaccharides from SI-III cells show that the Type III polysaccharide can be precipitated by Type III anticapsular serum without significant removal of the Type I component of the capsule and conversely, specific precipitation of the Type I capsular component is not accompanied by removal of the Type III component from solution.

TABLE III

Protective Effect of Type I and Type III Antiserum in Mice Infected with SI-III and with Mixtures of SI and SIII Pneumococci

Strain	Amount of culture injected	Serum injected	D/S*
	cc.		
SI-III	10^{-6}	Anti-SI	0/6
	10^{-6}	Anti-SIII	0/6
	10^{-6}	Normal	6/0
	10^{-6}	None	6/0
	10^{-7}	None	5/1
	10^{-8}	None	5/1

Viable units/cc. (undiluted culture): 4×10^8

Strains	Amount of culture injected	Serum injected	D/S*
	cc.		
SI + SIII	10^{-6}	Anti-SI	6/0
	10^{-6}	Anti-SIII	6/0
	10^{-6}	Normal	6/0
	10^{-6}	None	6/0
	10^{-7}	None	6/0
	10^{-8}	None	4/2

Viable units/cc. (undiluted culture): 3×10^8 (equal numbers of SI + SIII)

* D/S = died/survived.

A 200 cc. culture of SI-III cells was grown overnight after which time glucose was added to a final concentration of 1 per cent. During an additional 6 hours' incubation, the lactic acid formed was neutralized by the addition of 3 N NaOH. The cells were then removed by centrifugation and 1 volume of 95 per cent ethyl alcohol was added to the separated supernatant fluid. The suspension was stored overnight at 4°C. after which time, the precipitate formed was collected by centrifugation and dissolved in 10 cc. of normal salt solution. This solution served as the unfractionated capsular antigen. Aliquots thereof were allowed to

react with appropriate amounts of Type I or Type III anticapsular rabbit serum or with normal rabbit serum. All volumes were adjusted to give the same quantity of the original antigen in precipitin tests. The results are shown in Table IV.

On the bases of the independent variability of its capsular components, of the susceptibility of the Type III capsular component to the enzyme hydro-

TABLE IV

Independent Precipitability of Each Capsular Component of the SI-III Phenotype by Unitypic Capsular Antiserum

	Polysaccharide precipitated after treatment with normal rabbit serum									
	Dilution of antigen									
Serum	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
Anti-SI	+++	++	++	+	±	0	0	0	0	0
Anti-SIII	++++	+++	++	++	+	+	+	±	0	0
Normal	0	0	0	0						
	Polysaccharide precipitated after treatment with type I anticapsular rabbit serum									
	Dilution of antigen									
Serum	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
Anti-SI	±	0	0	0	0	0	0	0	0	0
Anti-SIII	++++	+++	+++	++	+	+	±	0	0	0
Normal	0	0								
	Polysaccharide precipitated after treatment with type III anticapsular rabbit serum									
	Dilution of antigen									
Serum	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
Anti-SI	++	+	+	±	0	0	0	0	0	0
Anti-SIII	0	0	0	0	0	0	0	0	0	0
Normal	0									

lyzing Type III pneumococcal polysaccharide, of the serologic cross-reactivity of its cells with Type I and Type III polysaccharides in precipitin tests in agar gels, and of the independent precipitability of its two capsular components, SI-III cells may be considered to produce two capsular polysaccharides.

Phagocytosis of SI-III Cells by Human Polymorphonuclear Leukocytes.—In Table V are shown the results of exposing SI-III cells to human polymorpho-

nuclear leukocytes in a smooth-walled system. Several facts of interest are demonstrable in these experiments. First, the persistence of the antiphagocytic effect of the Type III capsular component is demonstrable even in the presence of Type I anticapsular serum. That the Type I antiserum is fully effective is shown in the last line of the table. Second, the relatively small antiphagocytic effect of the Type I capsular component becomes apparent when the SI-III cells are exposed to the enzyme depolymerizing Type III polysaccharide together with normal rabbit serum. The efficacy of Type I antibody is definitely demonstrable in the presence of the same enzyme, however, by a further increase in phagocytosis.

The Relative Importance of the Two Capsular Components to the Virulence of SI-III Pneumococci.—Table VI shows the effect of several reagents upon the

TABLE V

Phagocytosis of SI-III Pneumococci by Human Polymorphonuclear Leukocytes in the Presence of Various Sera and of the Enzyme Hydrolyzing Type III Polysaccharide

Strain	Serum	Enzyme	Per cent PMN containing pneumococci	Average No. of pneumococci per PMN
SI-III	Anti-SI	Absent	8	0.36
	Anti-SIII	"	100	10.0
	Normal	"	4	0.08
	Anti-SI	Present	96	8.2
	Anti-SIII	"	96	8.0
	Normal	"	32	0.92
SI	Anti-SI	Absent	88	8.0

outcome of infection with SI-III cells in mice. It may be seen that each capsular polysaccharide contributes to the virulence of the infecting organism. Of interest is the role played by the Type I capsular component. Although the number of animals infected is small, it may be noted that the only animal infected with pneumococci manifesting binary capsulation and not receiving antiserum to survive was one treated with the enzyme depolymerizing Type III polysaccharide. Not shown in the table but noteworthy also is the fact that animals treated with a single injection of enzyme at the time of infection survived twice as long after inoculation as did untreated controls. Similar results have been observed in analogous experiments performed with cells of the binary capsular SIII-V phenotype. These observations are in accord with those derived from the study of phagocytosis of these strains. They suggest that the smaller Type I capsular component is able to protect the population of SI-III cells from complete elimination during the period that the enzyme is still hydrolyzing effectively the Type III capsular component but is unable by

itself to shift the balance of infection decisively in favor of the pneumococcus. Later, when the enzyme is no longer effective, regeneration of the Type III capsular component provides more effective protection against phagocytosis and permits rapid progression of the infection to a fatal outcome.

Properties of Antiserum Prepared with Vaccines of SI-III Pneumococci.—Rabbits immunized with vaccines of pneumococci manifesting binary capsulation form antibodies to each of the capsular components of such cells. The relative amount of antibody to each of the capsular antigens may vary during the course of immunization. Antisera to SI-III cells cause capsular swelling of SI, SIII, SI-III, and SIII-V cells. Shown in Table VII is the protective effect

TABLE VI

Effect of the Enzyme Hydrolyzing Type III Polysaccharide and of Unitypic Antisera on the Virulence of SI-III Pneumococci

Strain	Amount of culture injected	Serum injected	Enzyme injected	D/S
	cc.		*mg.*	
SI-III	10^{-6}	None	2	3/1
	10^{-6}	Anti-SIII	2	0/4
	10^{-6}	Anti-SIII	None	0/4
	10^{-6}	Anti-SI	"	0/4
	10^{-6}	None	"	3/0
	10^{-7}	"	"	2/0
SI	10^{-6}	"	2	4/0
SIII	10^{-6}	"	2	0/4
	10^{-6}	Anti-SIII	None	0/4

Viable units/cc. (undiluted culture): SI-III = 2×10^8; SI = 2×10^8; SIII = 3×10^8.

of such antiserum in mice infected with cells of each of the aforementioned phenotypes and with mixtures of SI and SIII cells. Recorded also in the table is the absence of protection against infection with pneumococcus Type V. Analogous results are observed in experiments concerned with the phagocytosis of pneumococci of several capsular types in the presence of Type I–III antiserum (Table VIII).

Precipitin tests in an agar gel (Fig. 3) disclose several facts. First, with Type I cells and Type I capsular polysaccharide, the anti-SI-III serum forms a continuous band of precipitate, and with Type III cells and Type III capsular polysaccharide a second and distinct band is formed. Second, these lines of precipitate are continuous with those formed by the antibody reacting with each of the capsular components of the SI-III cell. Third, there is no band of precipitate formed to indicate the presence of a single species of antibody

TABLE VII

Protective Effect of Type I-III Antiserum in Mice Infected with Pneumococci of a Variety of Capsular Types

Strain or strains	Amount of culture injected	Serum injected	D/S
	cc.		
SI-III	10^{-6}	Anti-SI-III	0/5
SI	10^{-6}	"	0/5
SIII	10^{-6}	"	0/5
SI + SIII	10^{-6}	"	0/5
SIII-V	10^{-6}	"	0/5
SV	10^{-6}	"	5/0
SIII + SV	10^{-6}	"	5/0
SI-III	10^{-6}	Normal	5/0
SI	10^{-6}	"	5/0
SIII	10^{-6}	"	5/0
SI + SIII	10^{-6}	"	5/0
SIII-V	10^{-6}	"	5/0
SV	10^{-6}	"	5/0
SIII + SV	10^{-6}	"	5/0
SI-III	10^{-7}	None	2/0
SI	10^{-7}	"	2/0
SIII	10^{-7}	"	2/0
SI + SIII	10^{-7}	"	2/0
SIII-V	10^{-7}	"	3/0
SV	10^{-7}	"	2/0
SIII + SV	10^{-7}	"	2/0

Viable units/cc. (undiluted culture): SI-III = 5×10^8; SI = 3×10^8; SIII = 2×10^8; SI + SIII = 2×10^8; SIII-V = 2×10^8; SV = 2×10^8; SIII + SV = 2×10^8.

TABLE VIII

Phagocytosis of Pneumococci of Several Types by Human Polymorphonuclear Leukocytes in the Presence of Type I-III Anticapsular Rabbit Serum

Strain	Serum	PMN containing pneumococci	Average No. of pneumococci per PMN
		per cent	
SI-III	Anti-SI-III	84	8.6
SI	"	60	9.4
SIII	"	92	8.1
SIII-V	"	72	7.4
SV	"	0	0
SI-III	Normal	0	0
SI	"	0	0
SIII	"	0	0
SIII-V	"	0	0
SV	"	0	0

reacting with both capsular components or the presence of a capsular component reacting with antibody in a fashion different from that of unitypic cells. The results are consistent with those presented earlier which indicate that cells manifesting binary capsulation produce two species of capsular polysaccharide each of which is closely similar if not identical with that of the corresponding unitypic pneumococcus.

In precipitin tests performed in tubes, Type I polysaccharide and Type III polysaccharide are each precipitated by antiserum prepared with a vaccine of

TABLE IX

Independent Precipitability of Capsular Antibodies from an Antiserum Prepared with a Vaccine of SI-III Pneumococci

	Polysaccharide precipitated by anti-SI-III serum before absorption											
	Dilution of antigen											
Antigen*	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
SI	++	++++	++++	++++	+++	++	+	+	±	±	0	0
SIII	++++	++++	++++	++++	+++	++	++	+	±	0	0	0
	Polysaccharide precipitated by anti-SI-III serum after absorption with SI cells											
	Dilution of antigen											
Antigen	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
SI	0	0	0	0	0	0	0	0	0	0	0	0
SIII	++++	++++	++++	++++	++++	+++	++	++	+	±	0	0
	Polysaccharide precipitated by anti-SI-III serum after absorption with SIII cells											
	Dilution of antigen											
Antigen	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	C
SI	+++	++++	++++	++++	++++	++++	+++	+	+	0	0	0
SIII	±	0	0	0	0	0	0	0	0	0	0	0

* Saline solution of purified Type I or Type III capsular polysaccharide in a concentration of 1 mg./cc. diluted as indicated.

SI-III cells. That two distinct anticapsular antibodies are present in such an antiserum can be demonstrated by the independent precipitability of each. When an antiserum prepared with a vaccine of SI-III cells is absorbed with either capsulated Type I or Type III cells, the corresponding anticapsular antibody is removed without significant alteration of the titer of antibody precipitating the other capsular component (Table IX). Analogous experiments in which precipitin reactions were carried out in an agar gel demonstrate the independent removal of one capsular antibody without alteration in the reaction of the other with appropriate antigens (Figs. 4 to 6).

Properties of Deoxyribonucleates of Pneumococci of the SI-III Phenotype.—To determine the nature of the genetic determinants of the capsular components of the SI-III cell, DNA was prepared from cultures of such cells in the usual manner. When cells of a non-capsulated pneumococcus (R36NC) derived originally from a strain of capsular Type II were exposed in the transforming system to DNA from SI-III cells, organisms of three different phenotypes were recovered: $S-_{III}$, SI and SI-III. No SIII cells were observed. The results indicate that there has been no major alteration in either of the genetic units concerned with the formation of the two capsular components of the cell manifesting binary capsulation during their residence within that cell and that each is able to engender expression of that character controlled by it when it is reintroduced as the sole genetic factor concerned with capsular formation into another pneumococcal cell.

DISCUSSION

Through the agency of the transformation reaction, it has been possible to isolate pneumococci manifesting binary capsulation of several phenotypes. Examination of cells of the SI-III phenotype in a number of experimental systems reveals that organisms of this type produce two distinct capsular polysaccharides which are closely similar if not identical to those produced by cells of the corresponding unitypic phenotypes. The evidence available suggests that the two types of polysaccharide are intermingled in the capsule in a random fashion. Study of cells of the SIII-V phenotype yields results of a similar nature.

Binary capsulation in pneumococcus provides a new basis for serologic cross-reactivity in this species. Cells manifesting binary capsulation react with unitypic antiserum to each of their capsular components and stimulate the formation of antibodies which will react with cells bearing either of the capsular antigens produced by cells of the binary capsular phenotype. Whether or not pneumococci manifesting binary capsulation exist in nature remains an unanswered question. Examination by genetic and serologic techniques of several groups of pneumococcal strains the capsular antigens of which show cross-reactivity has failed to result in the recognition in any of the strains of separable capsular antigens. It would appear more likely that the serologic interrelationships of these strains are based upon the presence of common chemical subgroups in their single capsular polysaccharides rather than upon their production of multiple types of capsular polysaccharide. For this reason the nomenclature of pneumococcal types proposed by Eddy (13) seems preferable to that proposed by Kauffmann and Lund (14).

These experiments with pneumococcus show many similarities to those performed with *H. influenzae* by Leidy, Hahn, and Alexander (6), and it appears probable that binary capsulation may be manifested by organisms of the

latter species. In each, however, it would appear to represent a product of the laboratory. This fact notwithstanding, binary capsulation provides a useful object for the study of a variety of biologic phenomena.

SUMMARY

Pneumococci manifesting binary capsulation have been produced through the agency of transformation reactions. Such cells produce two capsular polysaccharides which are closely similar if not identical with those produced by corresponding singly capsulated strains.

Binary capsulation in pneumococcus provides a new basis for serologic cross-reactivity in this species.

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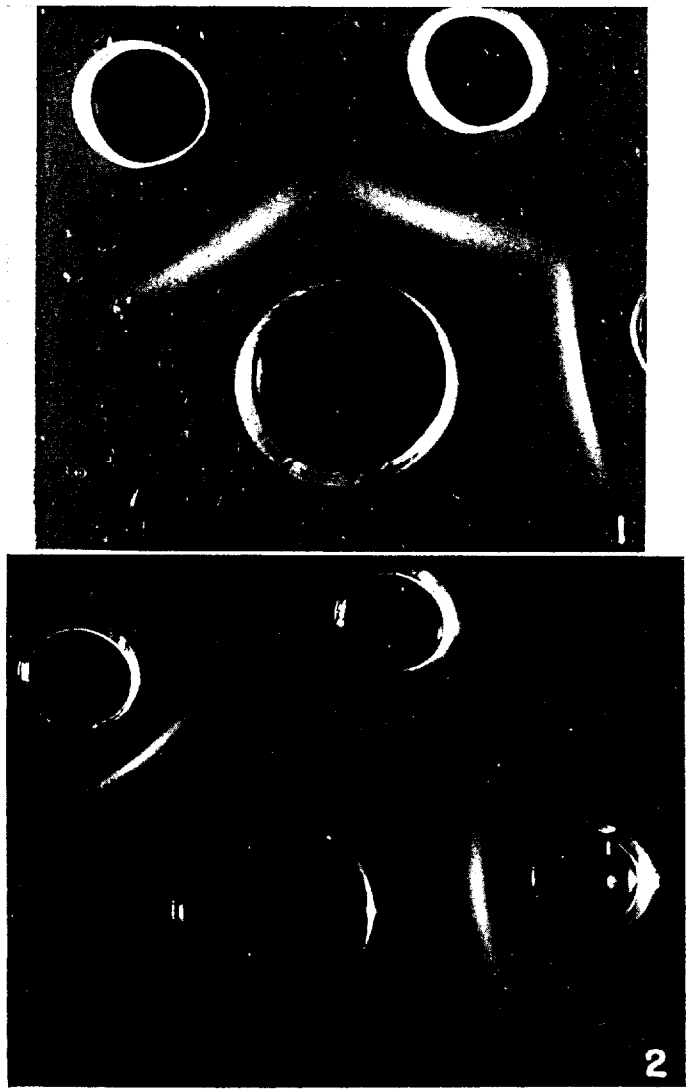
EXPLANATION OF PLATES

PLATE 32

FIG. 1. Reaction of Type III anticapsular antibody (A) with Type III cells (B), Type I-III cells (C), and Type III capsular polysaccharide (D).

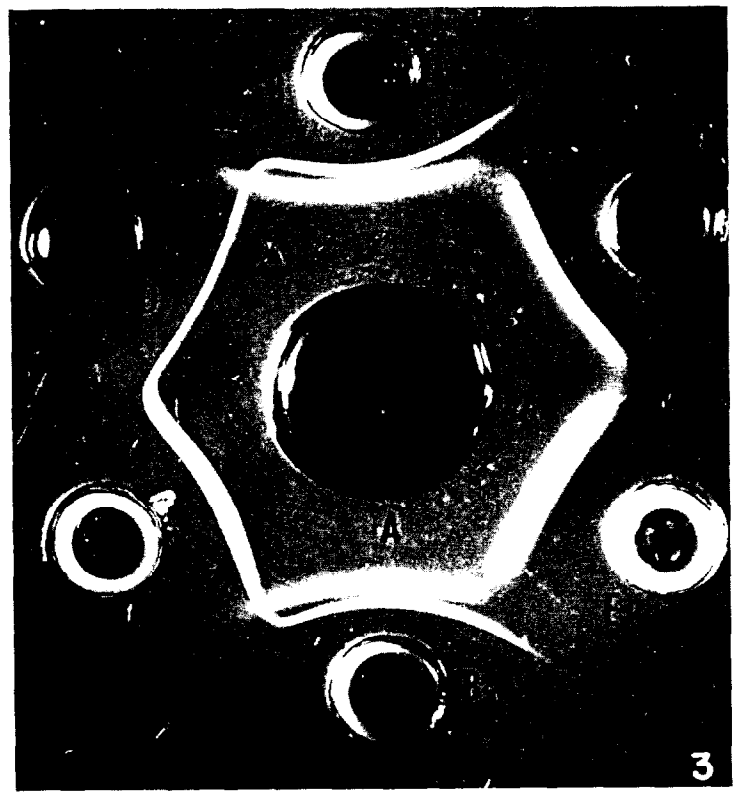
FIG. 2. Reaction of Type I anticapsular antibody (A) with Type I cells (B), Type I-III cells (C), and Type I capsular polysaccharide (D).



(Austrian and Bernheimer: Production of capsular polysaccharides. I)

PLATE 33

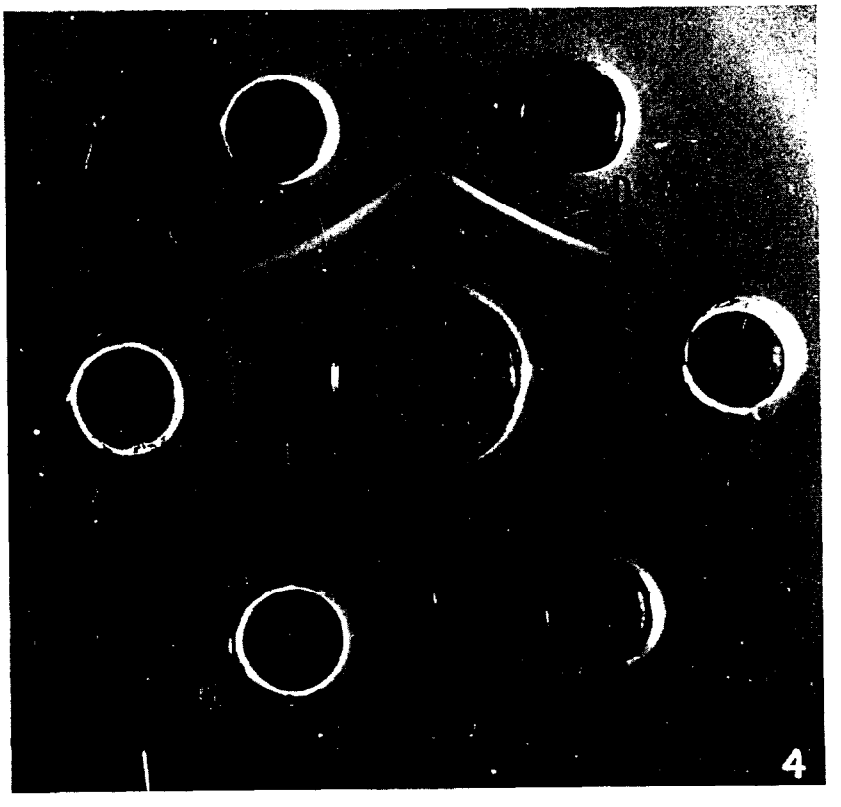
FIG. 3. Reaction of Type I-III anticapsular serum (A) with Type I-III cells (B), Type I cells (C), Type I polysaccharide (D), Type III cells (E) and Type III polysaccharide (F).



(Austrian and Bernheimer: Production of capsular polysaccharides. I)

PLATE 34

FIG. 4. Reaction of Type I-III anticapsular serum absorbed with Type III pneumococci (A), with Type I-III cells (B), Type I cells (C), Type I polysaccharide (D), Type III cells (E) and Type III polysaccharide (F).

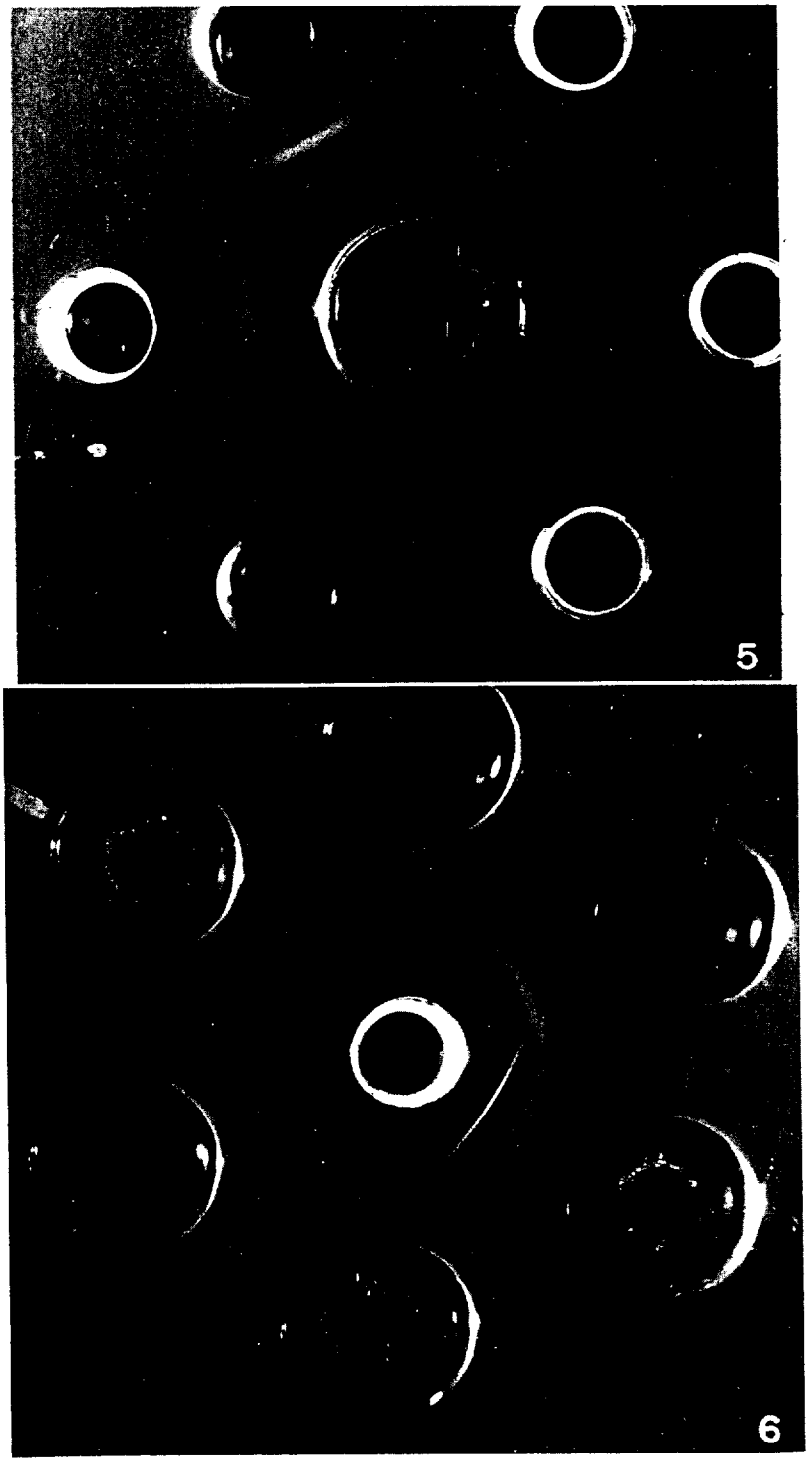


(Austrian and Bernheimer: Production of capsular polysaccharides. I)

PLATE 35

FIG. 5. Reaction of Type I-III anticapsular serum absorbed with Type I pneumococci (A), with Type I-III cells (B), Type I cells (C), Type I polysaccharide (D), Type III cells (E), and Type III polysaccharide (F).

FIG. 6. Reaction of Type I-III cells (A) with Type I-III antiserum (B), Type I-III antiserum absorbed with Type I pneumococci (C), Type III antiserum (D), Type I-III serum absorbed with Type III pneumococci (E), and Type I antiserum (F). Reactions between (A) and (F) failed to develop sufficiently to be visible in the photograph.



(Austrian and Bernheimer: Production of capsular polysaccharides. I)